

Heteromeric connexons in lens gap junction channels

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ABSTRACT Gap junction channels are formed by paired oligomeric membrane hemichannels called connexons, which are composed of proteins of the connexin family. Experiments with transfected cell lines and paired *Xenopus* oocytes have demonstrated that heterotypic intercellular channels which are formed by two connexons, each composed of a different connexin, can selectively occur. Studies by Stauffer [Stauffer, K. A. (1995) *J. Biol. Chem.* 270, 6768–6772] have shown that recombinant Cx26 and Cx32 coinfecting into insect cells may form heteromeric connexons. By solubilizing and subfractionating individual connexons from ovine lenses, we show by immunoprecipitation that connexons can contain two different connexins forming heteromeric assemblies *in vivo*.

Gap junctions are clusters of intercellular channels that permit the direct exchange of small metabolites, ions, and second messengers between the cytoplasm of adjacent cells (1), which play diverse roles in cellular signaling and growth regulation. Gap junction channels are formed by members of a family of proteins known as connexins (2). Connexin molecules oligomerize in the trans Golgi (3) into a membrane channel known as a connexon [hemichannel (4)], which is defined as homomeric when composed of the same connexin or heteromeric when composed of different connexins. Connexons in adjacent cells join head-to-head across a narrow extracellular “gap” to form intercellular channels, which are defined as homotypic when the same connexin comprises both connexons, and heterotypic when different connexins comprise each connexon of the pair. The expression of connexins is cell-type specific (5–7); however, multiple connexins are known to be expressed within the same cell type. For example, lens fibers express both Cx46 and Cx50 (8, 9), cardiac myocytes (10) and osteoclasts (11) express Cx45 and Cx43, and exocrine pancreas (12) and hepatocytes (13) express Cx32 and Cx26.

In myocardial cells, single channel conductance studies using dual whole-cell patch clamp methods have revealed more conductance states (14) than detectable members of the connexin family (12). These data, together with the demonstration of asymmetric voltage dependence in chicken cardiac myocytes (15), suggest the possibilities of either multiple conductance states for homomeric connexons in homotypic intercellular channels or the mixing of different connexins in heteromeric and heterotypic assemblies.

Heterotypic connexon interactions have been demonstrated to occur both in transfected cell systems (16, 17) and between paired *Xenopus* oocytes (18, 19), in some cases resulting in channel properties different from both parent connexins (20, 21). While many connexins are able to form heterotypic interactions, selectivity in connexin interactions has also been demonstrated. Cx40 and Cx43 have been shown to be incompatible both in the oocyte-pair expression system (22) and in transfected HeLa cells (16). In addition, antisense oligodeoxynucleotide treatment of the A7r5 smooth muscle cell line, which expresses both Cx40 and Cx43, results in the blockade

of some conductances and not others, indicating that these two connexons assemble into discrete intercellular channels (23).

Different connexins are known to colocalize in the same gap junctional maculae (8, 13, 24). Structural studies using scanning transmission electron microscopy have indicated that isolated gap junction maculae from rodent liver may contain heterotypic connexin channels formed by Cx26 and Cx32 (25). König and Zampighi (26) recently reported that they have isolated intercellular channels composed of bovine lens Cx44 and Cx50, although it could not be determined if these channels were heterotypic or heteromeric. Studies by Stauffer (27) have shown that recombinant rat Cx32 and Cx26, coinfecting into insect cell lines, can form heteromeric connexons. These studies provide the biochemical data that purified, recombinant connexons may be composed of both heteromeric and homomeric assemblies; however, the existence of heteromeric connexons *in vivo* has not been shown.

Three members of the connexin family are known to be expressed in the vertebrate lens. Cx43 is confined to the lens epithelial cells and to the differentiating lens fibers in the equatorial region of the organ (28, 29). The other two, Cx46 and Cx50, are expressed during the differentiation of, and persist in, mature lens fibers (8, 30, 31). Recent studies have shown, using immunocytochemistry, that Cx56 and Cx45.6, the avian counterparts of Cx46 and Cx50, are also coexpressed in the lens epithelium together with Cx43 (24, 32). Studies in the *Xenopus* oocyte-pair system have demonstrated lens connexin selectivity: Cx46 can form heterotypic interactions with both Cx43 and Cx50, but Cx43 cannot form heterotypic intercellular channels with Cx50 (33). This selectivity is in part conferred by the second extracellular domain.

Kistler *et al.* (34) have developed methods to isolate individual connexons from detergent-solubilized lens fiber cells and have used these preparations for reconstitution studies. In this investigation we have studied these isolated preparations using antisera specific for each connexin type to obtain direct biochemical evidence that individual connexons in the vertebrate lens are composed of two types of connexins which form heteromeric assemblies.

MATERIALS AND METHODS

Reagents. Fertilized, unincubated chicken eggs were obtained from SPAFAS (Norwich, CT) and were incubated for the desired times in a humidified 37°C incubator. Ovine and bovine eyes were obtained from a local abattoir. [³⁵S]Methionine (cell-labeling grade) was from New England Nuclear. Alkaline phosphatase-conjugated goat anti-rabbit IgG was from Promega. Octyl-β-D-glucopyranoside (8-Glu) and octylpolyoxyethylene (8-POE) were from Bachem. Glutamate decarboxylase was from Worthington. Tissue culture reagents were from GIBCO. Primary antisera have been previously characterized (8, 30, 32). Fetal calf serum was obtained from HyClone. The silver staining kit was from Pharmacia. All other chemicals were obtained from either Sigma or Fisher Scientific.

Abbreviations: Cx, connexin; 8-Glu, octyl-β-D-glucopyranoside; 8-POE, octylpolyoxyethylene.

Preparation of Gap Junction-Rich Lens Fiber Membrane.

The gap junction-rich lens fiber membranes were isolated as described (35). Briefly, the whole chicken lenses or cortical tissue from ovine or bovine lenses was lysed in the lysis buffer (5 mM Tris, pH 8.0/5 mM EDTA/EGTA) and crude membranes were pelleted at 25,000 rpm for 20 min (Beckman SW28 rotor). Membranes were extracted with 4 M urea/5 mM Tris, pH 9.5/5 mM EDTA/EGTA and followed by extraction with 20 mM NaOH and pelleting by centrifugation (25,000 rpm, 45 min, SW28). The membranes were washed with 5 mM Tris, pH 7.0/2 mM EDTA/EGTA/100 mM NaCl. Channel structures can be solubilized under low salt condition with various detergents such as 2% 8-Glu, 1% 8-POE, 0.5% Triton X-100, and 1% Nonidet P-40.

Metabolic Labeling and Immunoprecipitation. Intact lenses from 11-day embryonic chicken were dissected into culture medium (medium 199 plus 10% fetal calf serum) and metabolically labeled with [³⁵S]methionine (0.5 mCi; 1 Ci = 37 GBq) for 3 h and chased for 16 h in the presence of excess nonradioactive methionine (36). The detergent-solubilized membranes were immunoprecipitated with either anti-Cx45.6 or anti-Cx56 antibodies in the presence of 10 mM Hepes (pH 7.2) at 4°C overnight and then protein A-Sepharose beads were added for another 2 h. The beads were washed three times with wash solution (10 mM Hepes, pH 7.2/0.5% 8-POE) plus 1% bovine serum albumin (BSA) and twice with wash solution without BSA. The immunoprecipitated samples were isolated from beads by boiling in SDS sample buffer for 5 min. Immunoprecipitation of sucrose gradient fractions from ovine or bovine samples was performed with antibodies covalently conjugated to Sepharose beads. Anti-Cx46 antibody (411-416) was conjugated to protein A-Sepharose and anti-MP70 (Cx50) (6-4-B2-C6) antibody was conjugated to goat anti-mouse IgM agarose through a chemical cross linker, dimethyl pimelimidate, as described (37). Fractions (nos. 6–8) from sucrose sedimentation gradient were immunoprecipitated with the above conjugated antibodies in the presence of 10 mM Hepes, pH 7.2/0.5% 8-POE for overnight at 4°C and the beads were washed with wash solution.

SDS Gel Electrophoresis, Fluorography, Western Blots, and Silver Staining. Immunoprecipitates and fractions from sucrose gradients were analyzed on 10% SDS/polyacrylamide (SDS/PAGE) gels. Gels loaded with immunoprecipitated ³⁵S-labeled samples were processed for fluorography as described (38). Western blots of sucrose gradient fractions or immunoprecipitates were performed by probing with either anti-MP70 (Cx50) (1:10 dilution) or anti-Cx46 antibody (1:500 dilution). Primary antibodies were detected with alkaline phosphatase-conjugated goat anti-rabbit IgG for anti-Cx46 or goat anti-mouse Ig for anti-MP70 (Cx50) monoclonal antibody. The silver staining of immunoprecipitates on SDS/PAGE was performed with a kit (Pharmacia).

Sucrose Gradient Sedimentation Analysis. The detergent-solubilized membranes were fractionated on a linear gradient of 5–20% sucrose (wt/vol at 20°C) (4 ml total) in the presence of 10 mM Hepes, pH 7.2/0.5% 8-POE or 1% 8-Glu. Centrifugation was performed in a Beckman SW60 Ti rotor at 28,500 rpm for 18 h at 4°C, after which 200-μl fractions were collected.

RESULTS

Polyclonal anti-Cx45.6 and anti-Cx56 antibodies have been previously characterized and affinity-purified (32). These antibodies were used to detect possible physical interactions between these two connexins using immunoprecipitation methods (29). The unique solubility properties of lens fiber gap junctions maculae in nonionic detergents (34, 39) allowed us to obtain a population of soluble junctional channels, permitting a study of their molecular composition. Crude membranes

from metabolically labeled chicken lens were stripped with urea/alkali and solubilized in the presence of the nonionic detergent 8-POE (34). Fig. 1, lane 2, demonstrates that the anti-Cx45.6 antibody coimmunoprecipitated both Cx45.6 and Cx56 from these detergent-solubilized membranes. This result indicated that solubilized gap junctional channels of chicken lens contained both Cx45.6 and Cx56. Reciprocally, anti-Cx56 antibody also coimmunoprecipitated Cx45.6 (Fig. 1, lane 4). Lanes 1 and 3 showed that after denaturation with SDS, neither of the antibodies coimmunoprecipitated the cognate connexin, demonstrating the noncovalent association of these two connexins and the non-cross-reactivity of the antibodies. There are some minor uncharacterized proteins which associated with Cx45.6 and Cx56, evident in the coimmunoprecipitation samples (Fig. 1, lanes 2 and 4). Similar results were obtained with other nonionic detergents such as 2% 8-Glu, 0.5% Triton X-100, and 1% Nonidet P-40 [data not shown (34, 40)].

Since paired *Xenopus* oocytes injected with *in vitro* transcribed connexin mRNA are known to form functional gap junctional channels (18, 41), experiments were performed in the oocyte system to control for the possibility of nonspecific association or free exchange of connexins between connexin assemblies after the solubilization by detergent. Either Cx45.6 or Cx56 RNA along with [³⁵S]methionine was injected into *Xenopus* oocytes. ³⁵S-labeled products were solubilized by identical detergent conditions used above for immunoprecipitation and the different extracts were mixed together in order to permit possible subunit exchange. Following incubation, the mixed specimens were immunoprecipitated with either of the antibodies. Each antibody immunoprecipitated only its specific connexin; no coimmunoprecipitation of the cognate connexin was detectable (data not shown). These data demonstrated that the connexins did not exchange between multimeric connexin assemblies during detergent solubilization. This experiment also demonstrated that there was no cross-reactivity between the antibodies on nondenatured proteins.

The studies with solubilized chicken lens fiber membranes did not directly address whether the association between connexins was heterotypic or heteromeric. The additional proteins which associated with Cx45.6 and Cx56 resulted in a broad peak of connexin detectability across the sucrose gradients used to purify the connexons subtending the expected positions of connexons and intercellular channels (data not shown). We therefore took advantage of the work by Kistler *et al.* (34) and isolated samples from ovine lenses

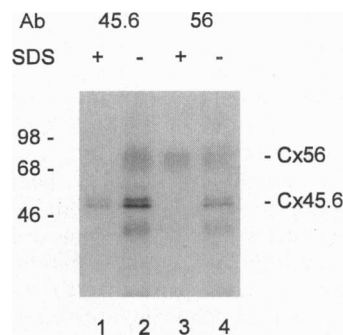


FIG. 1. Cx45.6 coimmunoprecipitates with Cx56. In the presence of 10 mM Hepes (pH 7.2), the urea/alkali stripped chicken lens membranes were solubilized by either 1% 8-POE (Bachem) (lanes 2 and 4) or 0.6% SDS (lanes 1 and 3), and unsolubilized material was pelleted at 25,000 rpm for 30 min (Beckman SW28 rotor) after 5 min of incubation at 23°C. The supernatants were immunoprecipitated with either anti-Cx45.6 (lanes 1 and 2) or anti-Cx56 (lanes 3 and 4) antibodies as described in the text. The immunoprecipitated samples were analyzed on SDS/PAGE and fluorography.

containing populations of single connexons. Lens connexons run as 9S particles, and intercellular channels or connexon pairs run at 16S. As has been shown in studies with Triton-solubilized connexons comprised of Cx43 from NRK cells, Cx43 monomers migrate with a sedimentation coefficient of 5S while connexons migrate with a sedimentation coefficient of 9S (3). In these experiments, we used either glutamate decarboxylase (EC 4.1.1.15; 310 kDa) or connexons composed of Cx43 (3) as markers for the connexons with a sedimentation coefficient of 9S. Based on protein standards and calculations, the sedimentation coefficient 9S migrated centered at fraction 7. 8-POE-solubilized ovine lens fiber membranes were centrifuged through 5–20% sucrose gradients. The resultant fractions were resolved using SDS/PAGE and then Western-blotted with the anti-MP70 (Cx50) monoclonal antibody. As shown in Fig. 2A, Cx50 concentrated into one major peak (fractions 6–8) at 9S, the position where connexons were previously shown to migrate (3, 34). Similarly, anti-Cx46 antibody also localized Cx46 into the same connexon-rich fractions (fractions 6–8) as those of Cx50 (Fig. 2B). Monomeric connexins prepared by SDS solubilization migrated at fractions 2–4 (5S) and connexon pairs prepared by 8-Glu solubilization (34) migrated at fractions 10–12 (16S) (data not shown).

We attempted to analyze the 9S fractions morphologically using negative stain electron microscopy, a method successfully used to visualize connexons isolated from rat liver (42, 43) and from ovine lens (34). While small areas of connexons in each specimen similar to those reported by Kistler *et al.* (34) could be visualized, the bulk of the material was highly aggregated. This aggregation was caused by the high ionic strength conditions which accompany sample preparation for negative staining; experimental aggregation and precipitation of the connexon samples occurred with minor increases in the salt concentration of the buffers (data not shown). Some aggregation may also have occurred directly

on the electron microscope grid during removal of detergent prior to application of the negative stain. This aggregation did not occur if the ionic strength was kept low. Connexon fractions which had been isolated from sucrose gradients and stored at 4°C for 1 month migrated precisely at the 9S position following resedimentation through fresh sucrose gradients (data not shown), thus excluding the possibility that the connexons aggregated in the sample before negative staining.

The sucrose gradient fractions were analyzed by immunoblots combined with immunoprecipitation approaches to examine whether the connexon population contained heteromeric connexons. To completely eliminate the cross-reactivity of secondary antibodies, anti-Cx46 antibody was covalently conjugated to protein A-Sepharose through a chemical cross-linking reaction (see *Materials and Methods*), and connexon-rich fractions were immunoprecipitated by anti-Cx46 antibody-conjugated beads. The immunoprecipitated samples were then resolved by SDS/PAGE and immunoblotted with anti-MP70 (Cx50) monoclonal antibody. Fig. 3, lane 2, shows that under nondenaturing conditions, anti-Cx46 immunoprecipitates contained Cx50, indicating the presence of these two connexins in a single connexon. Reciprocal experiments were performed in which connexon-rich fractions were immunoprecipitated with anti-MP70 (Cx50) conjugated to anti-mouse IgM agarose, followed by Western blotting of the immunoprecipitates with anti-Cx46 (Fig. 3, lane 3). These data further confirmed that single connexons were composed of both Cx46 and Cx50. Control experiments with SDS treatment prior to the immunoprecipitation showed neither of the cognate connexins were coimmunoprecipitated (Fig. 3, lanes 1 and 4). Similar experiments performed with bovine lenses also showed heteromeric connexons composed of bovine Cx46 and Cx50 (data not shown).

To exclude the possibility that other proteins might associate with connexins to form a connexon-like complex which migrated at 9S on sucrose gradient, we silver-stained the above immunoprecipitates from antibody-conjugated beads separated on SDS/PAGE. The silver-stained gels were quantitated by laser densitometry. Greater than 96% of total protein was represented by Cx46 and MP70 (Cx50), while the remaining

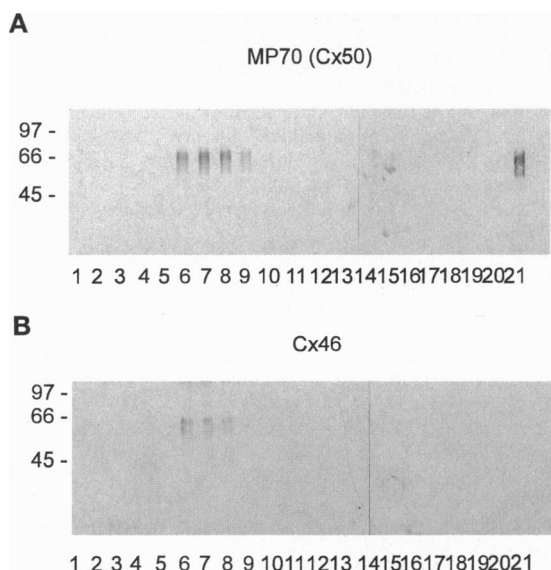


FIG. 2. Sucrose gradient analysis of ovine lens connexon isolation. Lens cortical tissue was dissected from decapsulated ovine lenses, lysed in the lysis buffer, and homogenized. The gap junction-rich membranes were isolated and solubilized (see text) in the presence of 1% 8-POE. 8-POE-solubilized specimens were separated by centrifugation (20,000 rpm, Beckman SW28 rotor, 30 min). The supernatants were fractionated on a linear gradient of 5–20% sucrose and 200- μ l fractions were collected. Each fraction was then boiled in 0.6% SDS, subjected to SDS/PAGE, and Western-blotted with either anti-MP70 (Cx50) (A) or anti-Cx46 (B) antibody. Both Cx50 and Cx46 migrate at 9S on the gradient, corresponding to the size of the connexon (3, 34).

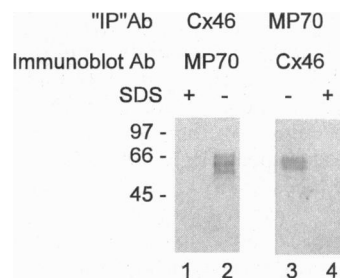


FIG. 3. Both ovine Cx46 and Cx50 are present in a single connexon. Fractions 6–8 from sucrose sedimentation gradients were immunoprecipitated with Sepharose-conjugated antibodies. For SDS-treated-samples, the fractions were boiled in 0.6% SDS before immunoprecipitation with these conjugated beads. Immunoprecipitated samples were subjected to SDS/PAGE and Western-blotted. The immunoprecipitates with anti-Cx46 were blotted using anti-MP70 (Cx50) antibodies (lanes 1 and 2) and those immunoprecipitated with anti-MP70 (Cx50) were blotted using anti-Cx46 antibodies (lanes 3 and 4). There are comparable levels of protein signal compared to those in Fig. 2, indicating that the immunoprecipitates do not correspond to a minor subset of the available connexons. In the presence of SDS (lanes 2 and 3), no bands were detected. Without SDS, Cx46 was found in the anti-MP70 (Cx50) immunoprecipitates and Cx50 was found in the Cx46 immunoprecipitates (lanes 1 and 4), indicating cooligomerization of these two connexins.

4% could be attributed to non-connexin contamination (data not shown).

DISCUSSION

The studies by Stauffer (27) provide biochemical evidence for heteromeric connexons using recombinant connexins coexpressed in baculovirus-infected insect cells. In her experiments, Cx32 (β_1 -connexin) and Cx26 (β_2 -connexin) both coelute from gel filtration columns in detergent-solubilized connexons from cells coinfecting with both connexins. Connexons from cells infected separately and mixed before detergent solubilization are clearly resolved on elution profiles from the columns, demonstrating that there is no detectable subunit exchange between connexons following detergent solubilization. The studies we report here provide direct evidence for the presence of heteromeric connexons in lens fibers *in vivo*. We have purified connexons solubilized under nondenaturing conditions using sucrose gradients and have shown that two connexins can be coimmunoprecipitated with either of two specific antibodies. Each antibody immunoprecipitated only its specific connexin following denaturation in SDS. We have immunoprecipitated detergent-solubilized mixtures of chicken connexins which had been translated separately in *Xenopus* oocytes, and we have not been able to detect any exchange. Thus, any possible exchange of monomeric connexins between the connexons in detergent solution was too slow relative to the time course of these experiments to account for the ability of the two proteins to coimmunoprecipitate. A limitation of this control experiment was that the connexins translated in the oocytes may not have been processed identically to those synthesized *in situ* by lens fibers and, hence, may not have had identical properties.

Unlike the connexons formed from Cx32 and Cx26, lens fiber connexons composed of MP70 (Cx50) and Cx46 aggregated with increased ionic strength, precluding the use of high-salt negative stain electron microscopy to quantitatively assess the monomeric form of the connexons. While limited fields of single connexons could be found similar to those published in the literature (34), the bulk of the grid-associated material was highly aggregated. That these aggregates formed during the process of negative staining was demonstrated by showing that the isolated connexons resedimented at 9S, the expected size of connexons, following 1 month storage at 4°C.

It was clear from the broad distribution of solubilized chicken connexins on sucrose gradients that the connexons were associated with other proteins in the detergent complexes. These proteins, together with the connexins, may be visualized in immunoprecipitates of radiolabeled specimens shown in Fig. 1, lanes 2 and 4. Unlike the chicken, however, ovine connexons ran as a sharp peak at 9S, indicating that these connexons are not associated with other proteins. Silver staining and densitometry of the ovine immunoprecipitates confirmed that non-connexin proteins comprised <4% of the total protein and thus were not in sufficient concentration to be stoichiometrically complexed with the connexins.

Our results demonstrate the *in vivo* presence of heteromeric connexons containing Cx46 and Cx50, indicating that previously observed selectivity of gap junctional channels is likely to be even more complicated than *in vitro* models. An acquisition of new regulatory properties of vertebrate lens gap junctions as a result of connexin heteromeric assembly may modify both the extent and the manner of intercellular communication between adjacent cells, such as selectivity and gating of the channels. Furthermore, since many (perhaps most) cells simultaneously express more than one type of connexin, assembly of heteromeric connexons could provide greater regulatory

flexibility and generate an increased complexity gap junction diversity throughout the body.

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- Bennett, M. V. L., Barrio, L. C., Bargiello, T. A., Spray, D. C., Hertzberg, E. & Saez, J. C. (1991) *Neuron* **6**, 305–320.
- Beyer, E. C., Paul, D. L. & Goodenough, D. A. (1990) *J. Membr. Biol.* **116**, 187–194.
- Musil, L. S. & Goodenough, D. A. (1993) *Cell* **74**, 1065–1077.
- Van Roy, F. & Mareel, M. (1992) *Trends Cell Biol.* **2**, 163–169.
- Clusin, W., Spray, D. C. & Bennett, M. V. (1975) *Nature (London)* **256**, 425–427.
- Haefliger, J.-A., Bruzzone, R., Jenkins, N. A., Gilbert, D. J., Copeland, N. G. & Paul, D. L. (1992) *J. Biol. Chem.* **267**, 2057–2064.
- Hennemann, H., Schwarz, H. J. & Willecke, K. (1992) *Eur. J. Cell Biol.* **57**, 51–58.
- Paul, D. L., Ebihara, L., Takemoto, L. J., Swenson, K. I. & Goodenough, D. A. (1991) *J. Cell Biol.* **115**, 1077–1089.
- Lo, W.-K., Mills, A. & Kuck, J. F. R. (1994) *Exp. Eye Res.* **58**, 189–196.
- Kanter, H. L., Laing, J. G., Beyer, E. C., Green, K. G. & Saffitz, J. E. (1993) *Circ. Res.* **73**, 344–350.
- Steinberg, T. H., Civitelli, R., Geist, S. T., Robertson, A. J., Hick, E., Veenstra, R. D., Wang, H.-Z., Warlow, P. M., Westphale, E. M., Laing, J. G. & Beyer, E. C. (1994) *EMBO J.* **13**, 744–750.
- Meda, P., Pepper, M. S., Traub, O., Willecke, K., Gros, D., Beyer, E., Nicholson, B., Paul, D. & Orci, L. (1993) *Endocrinology* **133**, 2371–2378.
- Nicholson, B., Dermietzel, R., Teplow, D., Traub, O., Willecke, K. & Revel, J. P. (1987) *Nature (London)* **329**, 732–734.
- Chen, Y.-H. & DeHaan, R. L. (1992) *J. Membr. Biol.* **127**, 95–111.
- DeHaan, R. L. & Chen, Y.-H. (1995) in *Intercellular Communication Through Gap Junctions*, eds. Kanno, Y. & Kataoka, K. (Elsevier, Amsterdam), pp. 187–200.
- Elfgang, C., Eckert, R., Lichtenberg-Fraté, H., Butterweck, A., Traub, O., Klein, R. A., Hülser, D. & Willecke, K. (1995) *J. Cell Biol.* **129**, 805–817.
- Moreno, A. P., Fishman, G. I., Beyer, E. C. & Spray, D. C. (1995) in *Intercellular Communication Through Gap Junctions*, eds. Kanno, Y., Kataoka, K., Shiba, Y., Shibata, Y. & Shimazu, T. (Elsevier, Amsterdam), pp. 405–408.
- Swenson, K. I., Jordan, J. R., Beyer, E. C. & Paul, D. L. (1989) *Cell* **57**, 145–155.
- Werner, R., Levine, E., Rabadan-Diehl, C. & Dahl, G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5380–5384.
- Rubin, J. B., Verselis, V. K., Bennett, M. V. L. & Bargiello, T. A. (1992) *Biophys. J.* **62**, 183–193.
- Barrio, L. C., Suchyna, T., Bargiello, T., Xu, L. X., Roginski, R. S., Bennett, M. V. L. & Nicholson, B. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8410–8414.
- Bruzzone, R., Haefliger, J.-A., Gimlich, R. L. & Paul, D. L. (1993) *Mol. Biol. Cell* **4**, 7–20.
- Moore, L. K. & Burt, J. M. (1994) *Am. J. Physiol.* **267**, C1371–C1380.
- Jiang, J. X., White, T. W. & Goodenough, D. A. (1995) *Dev. Biol.* **168**, 649–661.
- Sosinsky, G. E. (1995) in *Intercellular Communication Through Gap Junctions*, eds. Kanno, Y., Kataoka, K., Shiba, Y., Shibata, Y. & Shimazu, T. (Elsevier, Amsterdam), pp. 145–148.
- Konig, N. & Zampighi, G. A. (1995) *J. Cell Sci.* **108**, 3091–3098.
- Stauffer, K. A. (1995) *J. Biol. Chem.* **270**, 6768–6772.
- Beyer, E. C., Kistler, J., Paul, D. L. & Goodenough, D. A. (1989) *J. Cell Biol.* **108**, 595–605.
- Musil, L. S., Beyer, E. C. & Goodenough, D. A. (1990) *J. Membr. Biol.* **116**, 163–175.
- Kistler, J., Kirkland, B. & Bullivant, S. (1985) *J. Cell Biol.* **101**, 28–35.
- Grujters, W. T. M., Kistler, J., Bullivant, S. & Goodenough, D. A. (1987) *J. Cell Biol.* **104**, 565–572.

32. Jiang, J. X., White, T. W., Goodenough, D. A. & Paul, D. L. (1994) *Mol. Biol. Cell* **5**, 363–373.
33. White, T. W., Bruzzone, R., Wolfram, S., Paul, D. L. & Goodenough, D. A. (1994) *J. Cell Biol.* **125**, 879–892.
34. Kistler, J., Goldie, K., Donaldson, P. & Engel, A. (1994) *J. Cell Biol.* **126**, 1047–1058.
35. Bologna, M., Allen, R. & Dulbecco, R. (1986) *J. Cell Biol.* **102**, 560–567.
36. Musil, L. S., Cunningham, B. A., Edelman, G. M. & Goodenough, D. A. (1990) *J. Cell Biol.* **111**, 2077–2088.
37. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 521–523.
38. Jiang, J. X., Paul, D. L. & Goodenough, D. A. (1993) *Invest. Ophthalmol. Vis. Sci.* **34**, 3558–3565.
39. Goodenough, D. A. (1979) *Invest. Ophthalmol. Vis. Sci.* **18**, 1104–1122.
40. Kistler, J., Christie, D. & Bullivant, S. (1988) *Nature (London)* **331**, 721–723.
41. Dahl, G., Miller, T., Paul, D., Voellmy, R. & Werner, R. (1987) *Science* **236**, 1290–1293.
42. Stauffer, K. A., Kumar, N. M., Gilula, N. B. & Unwin, N. (1991) *J. Cell Biol.* **115**, 141–150.
43. Buehler, L. K., Stauffer, K. A., Gilula, N. B. & Kumar, N. M. (1995) *Biophys. J.* **68**, 1767–1775.